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Synthesis, Characterization, Theoretical prediction of activities and Evaluation of biological activities of Some Sulfacetamide based hydroxytriazenes

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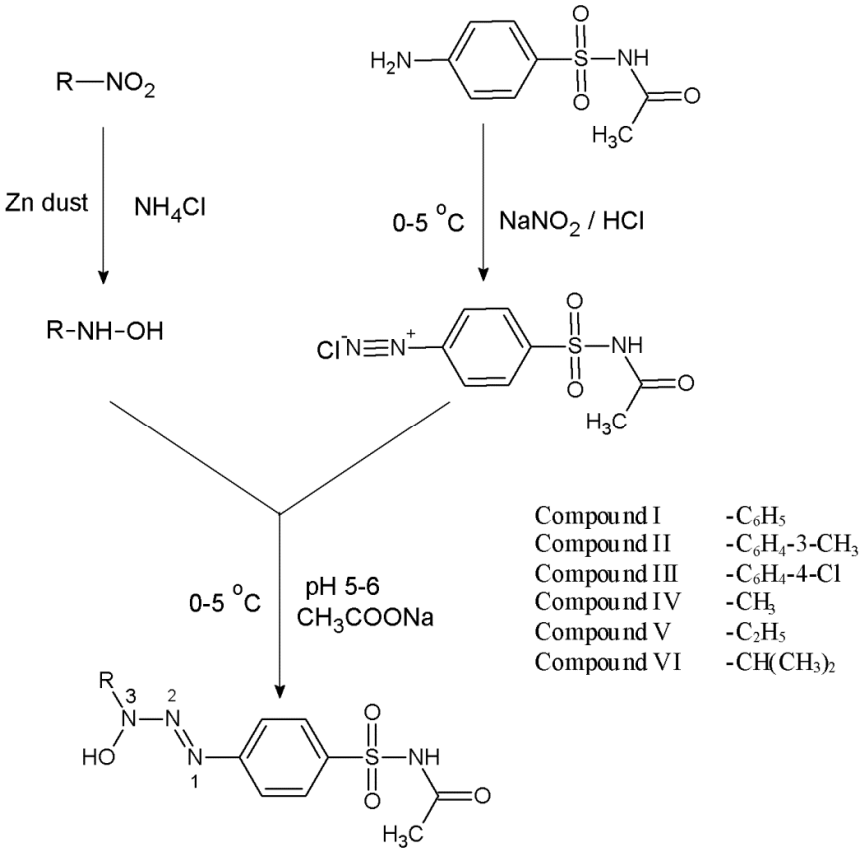
Six new *N* [(4-aminophenyl)sulfonyl]acetamide based hydroxytriazenes have been synthesized and characterized using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MASS spectral analysis. Further, their theoretical predictions for probable activities have been taken using PASS (Prediction of Activity Spectra for Substance). Although a number of activities have been predicted but specifically anti-inflammatory, anti-radical, anti-diabetic activities have been experimentally validated which proves that theoretical predictions agree with the experimental results. The object of the paper is to establish Computer Aided Drug Design (CADD) using our compounds.

Hydroxytriazenes have been extensively used as spectrophotometric reagents in our laboratory and elsewhere for determination of transition elements. Transition elements of first series extensively have been investigated using them as chelating agents. These reagents along with other spectrophotometric reagents have been reported by authors of our laboratory in form of reviews[1-2]. However, few hydroxytriazenes have been screened for their biological or pharmacological activities[3-8]. The earlier study[9-11] and theoretical prediction using PASS indicates that this series of compounds can be potential lead candidates for bioactivity if screened thoroughly. Based on this approach the present paper deals with CADD using known antibiotic sulfacetamide based hydroxytriazenes. Before planning experimental validation a theoretical prediction (PASS) was taken and out of many predicted activities only anti-inflammatory, anti-radical and anti-diabetic activities have been validated experimentally.

We have synthesized six hydroxytriazenes based on *N* [(4-aminophenyl)sulfonyl]acetamide [A.R. Grade purchased form Sigma (CAS No.: 144-80-9)] using method of Elkins and Hunter and further modified by Sogani and Bhattacharya[12-14]. The method involves reduction

of alkyl or aryl nitro compounds to obtain respective hydroxyl amine which is coupled with diazonium salt obtained from diazotization of amino group of sulfacetamide at 0-5℃, in 1:1 molar proportion in acetate buffer medium of pH 5.0. The compounds synthesized were purified and recrystallized using ethanol/ methanol. All the physicochemical characterization data have been provided in supporting information. The synthesis scheme is described below.



Scheme1. Synthesis of hydroxytriazenes based on Sulfacetamide

(Reagents- RNO_2; 0.03mol, Zn dust; 6.00g, NH_4Cl; 1.60g, Sulfacetamide; 0.02 mol, $NaNO_2$; 1.38 g)

PASS (Prediction of Activity Spectra for Substance) is a very good tool to predict theoretically activities of any molecule based on its chemical structure[15-17]. In the present work, theoretical predictions for twenty four hydroxytriazenes based on *N* [(4-aminophenyl)sulfonyl]acetamide as well as similar moiety were taken which included more than 300 activities (supporting information Table 1-4). Out of these, six hydroxytriazenes based on *N* [(4-aminophenyl)sulfonyl]acetamide were synthesized. However the activities predicted are probable and not to be validated experimentally but the activities are expressed in terms of Pa (the probability of compound being active) and Pi (the probability of

compound being inactive). Further in general Pa values more than 0.3 (30%) can be expected to have the predicted activities and compounds having Pa<0.3 may not possess the predicted activity.

For our compounds good probability for anti-inflammatory, anti-radical and anti-diabetic activities have been predicted as shown in supporting information table 1-4. Persual of tables, the predictions are in the range which warrents their experimental validation. Thus, it was planned to experimentally validate three activities namely anti-inflammatory, anti-radical and anti-diabetic activity.

Anti-inflammatory activity was determined by paw oedema method in rats as described by Winter *et al.*[18]. The rats were divided into nine groups. In group A, rats were given only vehicle (control group), in group B, rats were given carrageenan (0.1 ml of 1% mg /kg, bw, p.o.) whereas in Group C, animal were given carrageenan (0.1 ml of 1% mg /kg, bw, p.o.) single dose plus drug diclofenac (12.5 mg/kg bw, p.o.) and Group D rats were given carrageenan (0.1 ml of 1% mg /kg, bw, p.o.) plus synthesized hydroxytriazenes I-VI (200 mg/ kg/ day, bw, p.o.) orally. The mean paw volume at different time intervals was calculated and compared with control and the percentage inhibition was calculated.

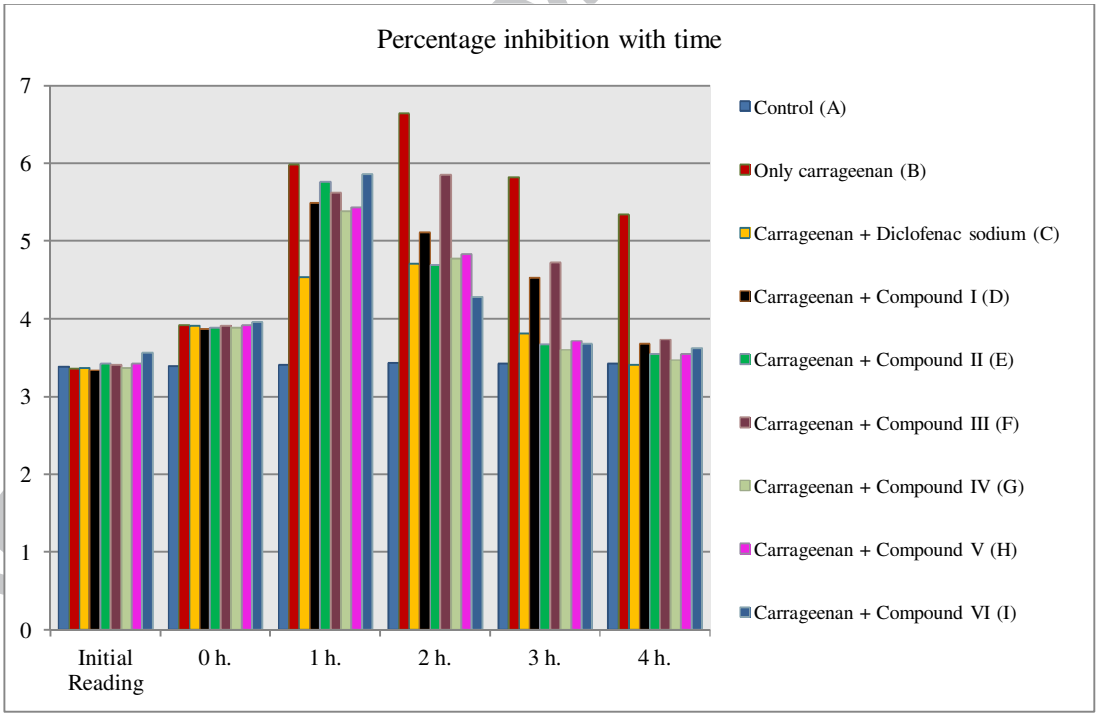


Figure 1. Anti-inflammatory activity of hydroxytriazenes

A perusal of figure 1 and table 7 (supporting information) reveals that out of six compounds studied compound no. VI (98.31%) produces highest percentage of inhibition after four hours

of treatment. Compound no. IV, II, V, III, and I show percentage of inhibition after four hours of treatment as 97.03%, 96.19%, 96.19%, 90.61% & 89.82% respectively. The results of experimental validation indicate that all the compounds show excellent anti-inflammatory activity and are in accordance to theoretical predication by PASS (the value of Pa ranging from 88 to 97.10%).

Hydroxytriazenes of a different series have been shown to possess anti-inflammatory activities in our earlier studies[9]. The inflammation is a protective response to cell injuries in animals which is manifested by clinical signs such as erythema, oedema, hyperalgesia, pain and loss of function at microscopic level. In the present study carrageenan induced acute inflammation model has been used and the results have been compared with standard drug Diclofenac sodium. The drug is categorized as a NSAID (Non-steroid anti-inflammatory drug) which acts at the periphery and not at CNS (Central Nervous System). Acting at the site of tissue injury these drugs block the synthesis of ecosanoids which finally blocks the cycloxygenase (COX) pathway. Thus, probable mechanism of action of carrageenan induced oedema is bi-phasic; the first phase is attributed to release of histamine-HT, kinins in the first hour, while the second phase is the release of prostaglandin like substance in 3-4 hours. Further the hydroxytriazenes exhibit structure dependent anti-inflammatory properties which are evidenced by all the six compounds through mechanism like NSAIDS.

The anti-radical activity screening of all synthesized hydroxytriazenes was done using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) modified method reported by Tripathi *et al.*[18]. DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

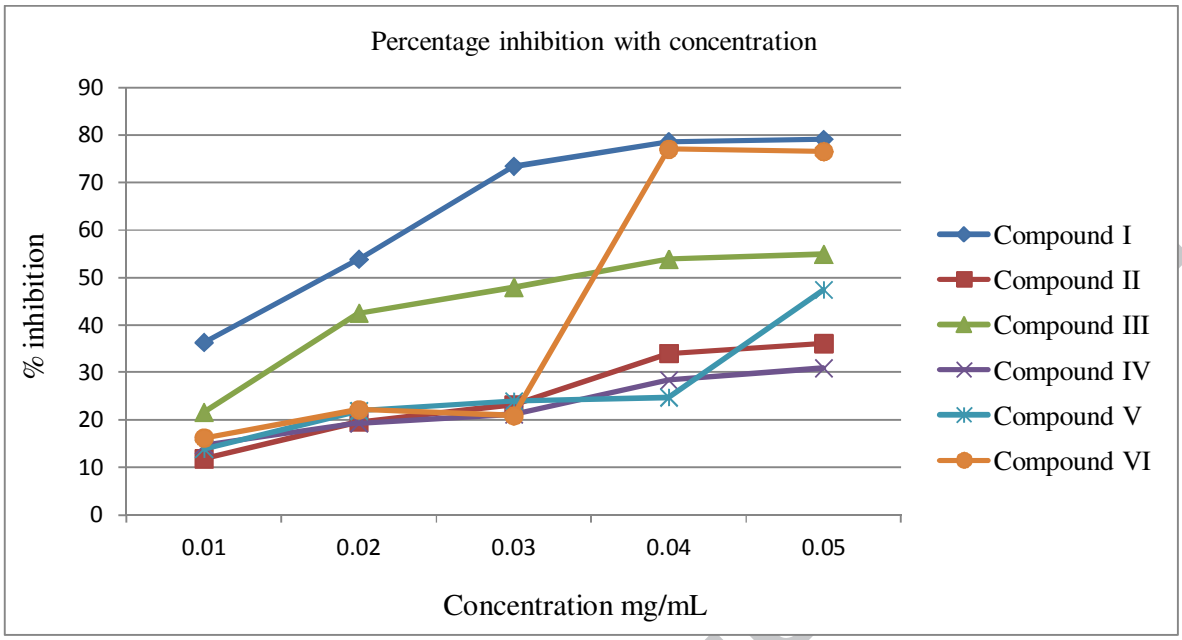


Figure 2 Anti-radical activity of hydroxytriazenes

The results shown in fig. 2 and table 8 (supporting information) indicate that the DPPH scavenging activity (antiradical activity) for mentioned hydroxytriazenes at 0.01-0.05 mg/mL are very significant as evidenced by IC_{50} values. It is further revealed that the best scavenging activity is shown by compound I whereas least active compound is compound IV. Compound III and VI are showing almost equal radical scavenging activity followed by compound II.

The DPPH model of scavenging is a standard and widely used method to evaluate anti-oxidant activity in a relatively short time. This is important to note here that, there is a great difference in antiradical and antioxidant activity as explained by some authors[19]. To clarify it can be mentioned that antiradical activity characterises the ability of any compound to react with free radical in a single reaction whereas antioxidant activity represents the ability to inhibit the process of oxidation. In case of hydroxytriazenes the antiradical activity is shown because of capacity of these molecules to donate hydrogen. DPPH is a stable free radical and accepts an electron of hydrogen radical to become a stable diamagnetic molecule[20].

For anti-diabetic activity of synthesized hydroxytriazenes α-glucosidase inhibition method reported by Tripathi *et al.*[21] was used. All the samples were run in triplicate and acarbose was taken as a standard reference compound. Several dilutions of primary solution (5 mg/mL DMSO) were made and assayed accordingly to obtain concentration of the test sample

required to inhibit 50% activity (IC_{50}) of the enzyme. Quantification was performed with respect to the standard curve of acarbose (Y = 1.271x + 71.39, R^2 = 0.989) and results were expressed as milligram of acarbose equivalent per mL of test sample. The percentage of enzyme inhibition by the sample was calculated by same formula as mentioned in antiradical activity.

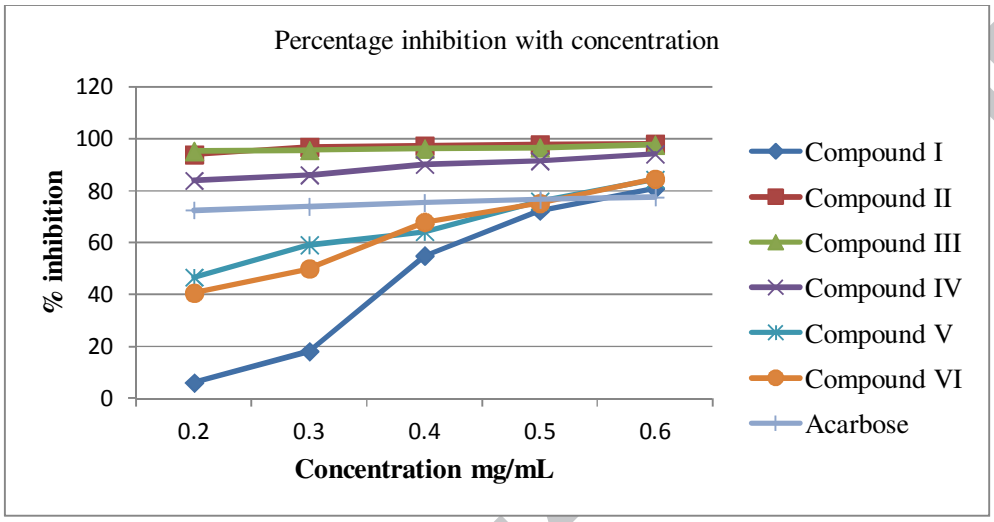


Figure 3 Anti-diabetic activity of hydroxytriazenes

Results of antidiabetic activities are shown in fig. 3 and table 10 (supporting information). Alpha-glucosidase inhibitive action is normally explained in terms of presence of enzyme α-glucosidase in small intestine. The enzyme is responsible for breaking dietary carbohydrates thus facilitating their absorption into the body. Inhibition of this enzyme allows less dietary carbohydrate available for absorption which in turn makes it less available in the blood following the meal. In case of hydroxytriazene the maximum inhibitory property is shown by compound V and least activity is exhibited by compound III. It can be concluded that the inhibitive action of hydroxytriazenes against α-glucosidase is structure correlated since the compounds having alkyl substituent on 3-position of hydroxytriazene (scheme 1) have enhanced activity whereas those with aryl substituent are least active. One more thing can be observed that the compounds having good anti-radical activity are also showing good anti-diabetic activity.

Reactive oxygen species are involved in diverse biological phenomena such as inflammation, carcinogenesis, ageing and atherosclerosis. The level of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker for oxidative stress is increased in the urine or the blood of type 2 diabetic patients. The levels of 8-OHdG and 4-hydroxy-2-nonenal (HNE)-were found elevated in the non obese type-2 diabetic rat model. These results indicate that the pancreatic β cells of rats

had oxidative stress and that chronic hyperglycemia might be responsible for the oxidative stress observed in the pancreatic β cells[22]. Insulin is secreted from β cell of islets of Langerhans of pancreatic gland. Suppression of insulin secretion produces hyperglycaemia. Further, cycloxygenase and thromboxanses are some of mediators associated with inflammation through the production of prostaglandins (PG). PG plays a major role in the production of oedema, erythema and pain in inflammation. PG is also said to be one of the most potent pyretic agent. PG can potentiate the carrageenan induced oedema and hyperalgesia in rats. Leukocytes appear to be responsible for the local release of cycloxygenase and thromboxane during carrageenan induced inflammation in rats[23]. Hydroxytriazenes and their derivatives significantly reduced carrageenan induced paw edema in rats. This explains that the compounds having good anti-inflammatory activities also exhibit fairly good anti-radical activities as well as anti-diabetic activities. The results show that all the three activities in case of hydroxytriazenes are good.

In conclusion, the paper describes computer aided drug designing and experimental validation of predicted activity. All the hydroxytriazenes (compound I-VI) synthesized have shown moderate to excellent anti-inflammatory, anti-radical and anti-diabetic activities. Thus hydroxytriazene can prove excellent candidates for multi targeted drugs since the compounds for present study are showing in addition to the activities reported in the paper many other activities which will be part of our future communication.

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